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(71) Applicant: PENTOSE PHARMACEUTICALS, INC. [US/US]; 45 Moulton Street, Cambridge, MA 02138 (US).

(72) Inventors: PURMAL, Andrei, A.; Apartment 11, 58 Jacqueline Road, Waltham, MA 02452 (US). ACKERMAN, Samuel, K.; 175 King's Grant Road, Weston, MA 02493 (US).

(74) Agent: CLARK, Paul, T.; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110-2214 (US).

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(54) Title: SOLID PHASE QUENCHING SYSTEMS

(57) Abstract

Methods of inactivating contaminants of a biological matrix are disclosed. The methods include the steps of: (a) contacting a biological matrix with an inactivating agent including an aziridino moiety, where a portion of the agent reacts with and inactivates the contaminant, and a portion of the agent remains unreacted; (b) contacting the product of step (a) with a solid support including at least 1 quenching moiety attached to the solid support through covalent bonds, under conditions and for a time sufficient to allow the unreacted agent to bond covalently to the quenching moiety; and (c) separating the solid support and the unreacted agent from the biological matrix, where the unreacted agent is attached to the solid support through covalent bonds.

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SOLID PHASE QUENCHING SYSTEMS

Background of the Invention

The invention relates to methods for quenching electrophiles.

The transmission of viral diseases (e.g., hepatitis A, B, and C, acquired immunodeficiency syndrome, and cytomegalovirus infections) by blood or blood products is a significant problem in medicine. Other biological compositions, such as mammalian and hybridoma cell lines, products of cell lines, milk, colostrum, and sperm, can also contain infectious viruses. Screening donor biological compositions for viral markers can help reduce the transmission of viruses to recipients, but many screening methods are directed to only a few discrete viruses, and are therefore incomplete, and may also be less than 100% sensitive. It is therefore important to inactivate viruses contained in donor blood, blood products, or other biological compositions.

A number of agents that are capable of inactivating viruses in blood have been developed. For example, ethyleneimine monomer and ethyleneimine oligomers (including dimers, trimers, and tetramers) are very effective viral inactivating agents. Methods for using ethyleneimine oligomers for inactivating viruses in biological compositions are described in U.S.S.N. 09/005,606 (filed January 12, 1998), hereby incorporated by reference. Ethyleneimine oligomers are themselves chemically active, and must therefore be rendered non-reactive before a product, such as blood, is used clinically. Typically, a viral inactivating compound, such as ethyleneimine dimer, is added to a biological composition to inactivate infectious viruses that might be present in the composition. A quenching agent is then added to inactivate the ethyleneimine dimer that remains after viral inactivation has taken place. The end result is a biological composition that is relatively free of infectious

viruses, but that is contaminated with quenched inactivating agent and with quenching agent.

Summary of the Invention

In one aspect, the invention features a method of inactivating a contaminant, such as a virus, of a biological composition; the method includes the steps of: (a) contacting the biological composition with an inactivating agent including an aziridino moiety, where a portion of the agent reacts with and inactivates the contaminant, and a portion of the agent remains unreacted; (b) contacting the product of step (a) with a solid support including at least 1 quenching moiety attached to the solid support through covalent bonds, under conditions and for a time sufficient to allow the unreacted agent to bond covalently to the quenching moiety; and (c) separating the solid support and the unreacted agent from the biological composition, where the unreacted agent is attached to the solid support through covalent bonds.

A preferred quenching moiety includes a nucleophilic moiety, such as a thiophosphate group; the thiophosphate group may be part of an internucleotide linkage of a oligonucleotide sequence. Preferably, the solid support contains at least 2 mmol/g thiophosphate moieties.

In another aspect, the invention features a method of inactivating a contaminant of a biological composition; the method includes the steps of: (a) contacting the biological composition with an inactivating agent including an aziridino moiety, where a portion of the agent reacts with and inactivates the contaminant, and a portion of the agent remains unreacted; (b) contacting the product of step (a) with a quenching agent under conditions and for a time sufficient to allow the quenching agent to quench and to bond covalently to the unreacted inactivating agent; (c) contacting the product of step (b) with a solid support including at least 1 aldehyde moiety covalently bonded to the solid

support, under conditions and for a time sufficient to allow the quenched inactivating agent to bond covalently to the aldehyde moiety; and (d) separating (i) the solid support, (ii) the quenched inactivating agent, and (iii) the quenching agent from the biological composition, where the inactivating agent and the quenching agent are attached to the solid support through covalent bonds. Preferably, the solid support contains at least 2 mmol/g aldehyde moieties attached to it.

In yet another aspect, the invention features a method of quenching an electrophile; the method includes contacting the electrophile with a solid support including at least 1 thiophosphate moiety attached to the solid support through covalent bonds, under conditions and for a time sufficient to allow the electrophile to bond covalently to the thiophosphate moiety. The solid support preferably includes at least 2 mmol/g thiophosphate moieties, and more preferably includes at least 100 mmol/g thiophosphate moieties. In preferred methods, a plurality of the thiophosphate moieties are substituted with at least one C_{1-12} saturated or unsaturated hydrocarbon skeleton that is unsubstituted or has between 1 and 4, inclusive, substituents, independently selected from the group consisting of hydroxyl, amino, cyano, and azido moieties. Preferably, the electrophile includes an aziridino moiety. For example, the electrophile may be ethyleneimine or an oligomer of ethyleneimine.

In another aspect, the invention features a method of removing a viral inactivating agent from a biological composition; the method includes the steps of: (a) contacting the inactivating agent with a quenching agent that is attached to a solid support through covalent bonds; and (b) removing the inactivating agent, the quenching agent, and the solid support from the biological composition. Preferably, step (a) includes contacting the inactivating agent with the quenching agent under conditions and for a time sufficient to allow covalent bonds to form between the inactivating agent and the quenching

agent. A preferred quenching agent includes a nucleophilic moiety, such as a thiophosphate moiety.

In still another aspect, the invention features a method of inactivating a contaminant of a biological composition. The method includes the steps of: (a) contacting the biological composition with an inactivating agent including an aziridino moiety or a haloderivative salt thereof, where a portion of the agent reacts with and inactivates the contaminant, and a portion of the agent remains unreacted; (b) contacting the product of step (a) with a quenching agent which includes a quenching moiety under conditions and for a time sufficient to allow the inactivating agent to bond covalently to the quenching moiety; and (c) separating the quenching agent and the quenched inactivating agent from the biological composition.

In still another aspect, the invention features a method of inactivating a contaminant of a biological composition. The method includes the steps of: (a) contacting the biological composition with an inactivating agent including an aziridino moiety or a haloderivative salt thereof, where a portion of the agent reacts with and inactivates the contaminant, and a portion of the agent remains unreacted; (b) contacting the product of step (a) with a quenching moiety, attached to a separation moiety through covalent bonds, under conditions and for a time sufficient to allow the inactivating agent to bond covalently to the quenching moiety; and (c) separating the separation moiety, the quenching moiety and the quenched inactivating agent from the biological composition.

A preferred separation moiety is selected from the group consisting of a bead, a resin, an antibody, and a biotin molecule. The composition of the second aspect preferably also includes a reporter moiety selected from the group consisting of a UV adsorbing moiety and a fluorescent moiety.

In yet another aspect, the invention features a method of quenching an electrophile. The method includes contacting the electrophile with a

composition including a thiosulfate or thiophosphate moiety attached to a separation moiety, under conditions and for a time sufficient to allow the electrophile to bond covalently to the thiosulfate or thiophosphate moiety. Preferably, the electrophile includes an aziridino moiety or a haloderivative salt thereof. For example, the electrophile may be ethyleneimine, an ethyleneimine oligomer, a haloderivative salt of ethyleneimine, or a haloderivative salt of an ethyleneimine oligomer. A preferred electrophile is N-acetylethyleneimine. Preferably, the separation moiety is selected from the group consisting of a bead, a resin, an antibody, and a biotin molecule. The composition may further include a reporter moiety, such as a UV adsorbing or fluorescent moiety.

In another aspect, the invention features a method of removing a viral inactivating agent from a biological composition. The method includes the steps of: (a) contacting the inactivating agent with a quenching moiety that is coupled to a separation moiety selected from the group consisting of a bead, a resin, an antibody, and a biotin molecule; and (b) removing the inactivating agent, the quenching moiety, and the separation moiety from the biological composition. Preferably, step (a) includes contacting the inactivating agent with the quenching moiety under conditions and for a time sufficient to allow covalent bonds to form between the inactivating agent and the quenching moiety. A preferred quenching moiety includes a nucleophilic moiety, such as a thiosulfate or thiophosphate moiety.

In another aspect, the invention features a compound which includes (a) a separation moiety; and (b) a thiosulfate or thiophosphate moiety. Preferably, the separation moiety is selected from the group consisting of a bead, a resin, an antibody, and a biotin molecule. The compound may further include a reporter moiety, such as a UV adsorbing or fluorescent group. The thiophosphate moiety may be part of an internucleotide linkage of an oligonucleotide sequence.

Inactivating agents of the invention may be, for example, ethyleneimine, an ethyleneimine oligomer, a haloderivative salt of ethyleneimine, or a haloderivative salt of an ethyleneimine oligomer. A preferred inactivating agent is N-acetylethyleneimine. The biological composition may be selected from the group consisting of whole mammalian blood, purified or partially purified blood proteins, blood cell proteins, milk, saliva, blood plasma, platelet-rich plasma, a plasma concentrate, a precipitate from any fractionation of plasma, a supernatant from any fractionation of plasma, a serum, a cryoprecipitate, a cryosupernatant, a cell lysate, a mammalian cell culture, a mammalian culture supernatant, a placental extract, a product of fermentation, a platelet concentrate, a leukocyte concentrate, semen, red blood cells, and a recombinant protein-containing composition produced in a transgenic mammal. Preferably, the biological composition is whole human blood or human blood plasma. The contaminant may be a virus.

Preferred quenching moieties include a nucleophilic moiety, such as a thiophosphate or thiosulfate moiety; the thiophosphate moiety may be part of an internucleotide linkage of an oligonucleotide sequence.

By "biological composition" is meant a composition that contains biological macromolecules, such as proteins, nucleic acids, lipids, and carbohydrates.

By "quenching moiety" or "quenching agent" is meant a moiety or an agent that is capable of reacting with, and thereby reducing the reactivity of, an electrophilic compound.

By "reporter moiety" is meant a UV adsorbing or fluorescent group which is added to the quenching agent for the monitoring of removal of the quenching agent and the quenched inactivating agent.

By "separation moiety" is meant a solid phase moiety which confers to a compound at least one property which allows for its separation from most

other compounds in a biological composition. Preferred properties include selective high-affinity to a compound not normally present in the biological composition, the ability of the moiety to be separated from the biological composition through filtration, centrifugation, or placement in a magnetic field. A bead, a resin, an antibody, and a biotin molecule are each a preferred separation moiety.

The invention provides new methods for the quenching of viral inactivating agents and the subsequent removal of the quenching and inactivating agents from a biological composition. This method results in a biological composition that is relatively free not only of contaminating viruses, but also relatively free of quenched (i.e., non-reactive) inactivating agent and unreacted quenching agent. The invention provides methods which are compatible with methods of removing solvent and detergent from protein-containing preparations which are virally-inactivated by a solvent/detergent method.

Other features and advantages of the invention will be apparent from the following description and from the claims.

Brief Description of the Drawings

Fig. 1 is a scheme showing the preparation of a solid-phase quenching agent that contains a thiophosphate group.

Fig. 2 is a scheme showing the preparation of a solid-phase quenching agent that contains multiple thiophosphate groups.

Fig. 3 is a scheme showing the quenching of an aziridino compound with a thiophosphate group that is covalently bonded to a solid support.

Fig. 4 is a scheme showing the quenching of an oligoethyleneimine with a solid-phase quenching agent containing phosphothio diester and phosphothio monoester groups.

Fig. 5 is a graph showing the quenching of ethyleneimine dimer in whole CPD human blood using a solid-phase quenching agent.

Fig. 6 is a graph showing the quenching of ethyleneimine dimer with a solid phase quenching agent containing thiophosphomonoester groups.

Fig. 7 is a graph showing the quenching of ethyleneimine dimer with solid phase quenching agent containing thiophosphodiester and thiophosphomonoester groups.

Detailed Description

The invention provides general methods for quenching electrophiles with quenching agents, such as thiophosphate moieties, that are attached to solid supports. Examples of such solid phase quenching systems are shown in Figs. 1, 2, 3, and 4. A solid support containing a single thiophosphate moiety is shown in Fig. 1, and supports having a number of thiophosphate moieties attached to the support by covalent bonds are shown in Figs. 2, 3, and 4.

The number of quenching moieties that can be attached to a support depends, in part, on the number of functional groups on the surface of the support. The total number of quenching moieties also depends on the number of quenching moieties attached to each functional group. For example, a polymeric support may contain 2 mmol/g hydroxyl groups; each of these hydroxyl groups can have as many as 150 thiophosphate groups attached to it. The solid support would therefore have 300 mmol/g thiophosphate groups.

The thiophosphate groups can be attached directly to the solid support, or they can be attached to the solid support through linkers. The linkers may have as many as 100 atoms. An example of a linking group is shown in Fig. 1; in this figure, the thiophosphate moiety is covalently bonded to an ethylene linker, which in turn is bonded to the solid support.

The invention also provides general methods for quenching

electrophiles with nucleophilic quenching agents, such as thiosulfate or thiophosphate moieties, that are modified so as to allow for the removal of the electrophile and the quenching agent from a biological composition.

A method of removal of quenching agent and quenched virus inactivating agent from a biological composition is through the use of nucleophiles, such as thiosulfate or thiophosphate groups, which are attached to a solid support or have attached to them a second moiety (the separation moiety) which supplies particular properties to the quenching agent, such that the quenching agent can be completely and reliably separated, along with the quenched inactivating agent, from the biological composition. These modified quenching compounds can react with and quench electrophiles such as an ethyleneimine oligomer or N-acetylethyleneimine. This method has the added advantage that it is compatible with methods to remove solvent and detergent from protein-containing preparations which are virally-inactivated by the Solvent/Detergent procedure described by Budowsky et al., U.S.S.N. 09/005,719. The Solvent/Detergent method of virus activation is compatible with the virus inactivation by compounds such as ethyleneimine monomer and ethyleneimine oligomer. Thus, one can perform two methods of viral inactivation in sequence. Alternatively, the inactivation of viruses through the use of, for example, ethyleneimine oligomer, followed by quenching and removal of the quenching and inactivating agents using the methods described herein, can be performed without the use of the Solvent/Detergent method. It is also advantageous that the quenching agent be easily detectable in order to monitor its removal. This can fulfilled with the addition of thymidine, which is readily detected by its adsorbance of 260 nm light.

A variety of materials can be used as the solid support. Examples of such materials include polymers (e.g., polyvinyl chloride, polytetrafluoroethylene), nylons (e.g., Dacron®), polyacrylamide pads deposited

on solid surfaces, silicon, silicon-glass, and glass. The solid supports may be in any form suitable for use in the invention. For example, the solid supports may be in the form of beads, rods, or films; alternatively, the solid supports may be in the form of permeable and semi-permeable membranes. The solid support may be contained within a filtration device, such as a column or a cartridge.

Solid supports containing hydroxyl groups are commercially available, for example, from Argonaut Technologies, San Carlos, CA. If the solid support does not have functional groups, such as hydroxyl or amino groups, on its surface, it can be derivatized. Any number of agents may be used to functionalize the solid support, as long as the end product is a solid support with a functional groups attached.

The thiophosphate groups used in the invention may be substituted with one substituent (e.g., [X]-OP(=S)$(OH)_2$, also referred to as a thiophosphomonoester), substituted with two substituents (e.g., [X]-OP(=S)(OH)(OAlk), a thiophosphodiester), or substituted with three substituents (e.g., [X]-OP(=S)$(OAlk)_2$, a phosphothiotriester), wherein X is a solid support or a separation moiety. The substituent may be, for example, a linear, branched, or cyclic saturated or unsaturated hydrocarbon with one to forty carbons, a benzyl group, a polycyclic aromatic group, an unsubstituted alkyl group, or an alkyl group substituted with hydroxyl, amino, azido, or cyano groups.

Polythiophosphate moieties (i.e., moieties having two or more adjacent phosphate groups) can also be used in the invention. For example, guanosine diphosphate (GDP) or guanosine triphosphate (GTP), in which one or more of the phosphate groups is a thiophosphate group, may be used in the invention. In the case of guanosine diphosphate, one or both phosphate groups may be thiophosphate groups. In the case of guanosine triphosphate, one, two, or all three of the phosphate groups may be thiophosphate groups. GDP or

GTP may be attached to the separation moiety, for example, at the 2' or the 3' hydroxyl group or to the heterocyclic base.

The compositions of the invention can be prepared as described below in the Examples. They can also be prepared using other standard synthetic techniques of oligonucleotide synthesis, such as those described in *Oligonucleotides and Analogs: A Practical Approach* (Eckstein ed., IRL Press 1991).

The quenching systems of the invention can be used as follows. A viral inactivating agent, such as an ethyleneimine oligomer, is added to a biological composition, as described in Budowsky, U.S.S.N. 08/855,378 and Budowsky et al., U.S.S.N. 09/005,606. At the end of the time necessary for viral inactivation, the biological composition is contacted with quenching agent, a compound containing one or more thiosulfate or thiophosphate moieties attached to a separation moiety. The biological composition and the quenching agent are allowed to remain in contact for at least one hour, at room temperature and a pH of 7.0. A 10-fold excess of thiosulfate or thiophosphate groups per equivalent of ethyleneimine oligomer is used.

The thiosulfate or thiophosphate moieties react with the highly reactive moieties of the ethyleneimine compounds or their haloderivative salts, and become covalently linked to these compounds. When the coupled thiosulfate or thiophosphate moieties are removed from the biological composition, therefore, the quenched ethyleneimine compounds are removed as well. The end result is a biological composition that is substantially free of infectious viruses, quenched ethyleneimine compounds, and quenching agent.

For example, a biological composition containing the inactivating agent ethyleneimine dimer can be quenched with sodium thiosulfate. Methods for inactivating viruses in biological compositions and quenching with thiosulfate are well known in the art and are described, for example, in

Budowsky, U.S.S.N. 08/835,446. The thiosulfate reacts with the aziridine ring and remains covalently bound to the quenched ethyleneimine dimer. A solid support having aldehyde groups can then be added to the reaction mixture. Such supports are commercially available from a number of sources, including Argonaut Technologies. The support and the composition are allowed to remain in contact for at least one hour, at room temperature and a pH of 7. A 10-fold excess of aldehyde groups per equivalent of ethyleneimine dimer is used. The aldehyde moieties react with the primary amino groups of the dimers to form relatively stable imine complexes; the quenched inactivating agent, as well as the thiosulfate moiety, therefore remains covalently bonded to the solid support. The solid support can be removed from the composition, for example by filtration, along with the quenched inactivating agent and the quenching agent.

In another example, the solid phase quenching systems of the invention can be used as follows. A viral inactivating agent, such as an ethyleneimine oligomer, is added to a biological composition, as described in Budowsky, U.S.S.N. 08/855,378 and Budowsky et al., U.S.S.N. 90/005,606. At the end of the time necessary for viral inactivation, the biological composition is contacted with a solid support containing thiophosphate groups. The composition and the solid support are allowed to remain in contact for at least one hour, at room temperature and a pH of 7. A 10-fold excess of thiophosphate groups per equivalent of ethyleneimine oligomer is used.

The support can be added to the composition in the form of particles; these particles can be removed, for example, by filtration after the inactivating agent is quenched. Alternatively, the biological composition can be passed through a filtration device, such as a column that contains support-bound thiophosphate groups.

Another way to obtain biological compositions free of infectious

viruses, quenched inactivating agent, and quenching agent is to inactivate viruses in the compositions with an inactivating agent, such as ethyleneimine dimer, as described above. After the viruses have been inactivated, the inactivating agent can be quenched with a traditional quenching agent, such as sodium thiosulfate. A solid support containing an electrophilic group can then be added; reactive groups on the support form covalent bonds with the quenched inactivating agent. The quenched inactivating agent and the quenching agent can then be easily removed from the composition, as they are attached to the solid support through covalent bonds.

The biological composition may include any of a number of substances. Examples of compositions include whole mammalian blood, purified or partially purified blood proteins, blood cell proteins, milk, saliva, blood plasma, platelet-rich plasma, a plasma concentrate, a precipitate from any fractionation of plasma, a supernatant from any fractionation of plasma, a serum, a cryoprecipitate, a cryosupernatant, a cell lysate, a mammalian cell culture, a mammalian culture supernatant, a placental extract, a product of fermentation, a platelet concentrate, a leukocyte concentrate, semen, and red blood cells. Other biological compositions include those containing recombinant proteins produced in transgenic mammals. For example, the biological composition may include a protein that has been expressed in the milk of a transgenic mammal. Methods for producing such proteins are described, for example, in Wright et al., *BioTechnology* 9:830-834 (1991) and the references cited therein.

There now follow particular examples that describe the preparation of quenching systems of the invention and the use of these systems to quench viral inactivating agents. These examples are provided for the purpose of illustrating the invention, and should not be construed as limiting.

Example 1: Preparation of a solid-phase quenching agent that contains a thiophosphate group

The preparation of a solid phase quenching agent of the invention is described in Fig. 1. As shown there, a solid support (designated with a square) containing a hydroxyl group is derivatized with a phosphorylating agent. The phosphite group of the phosphorylated solid support is oxidized to form a thiophosphate ester, which is cleaved with acid to provide a thiophosphate moiety. The product is a thiophosphate moiety that is attached to a solid support through covalent bonds.

Example 2: Preparation of a solid-phase quenching agent that contains multiple thiophosphate groups

The preparation of a solid support with a high loading of thiophosphate groups is shown in Fig. 2. As shown there, ethylene glycol is derivatized to yield a monomer (I) containing a phosphoramidite group. This monomer is polymerized, using a solid support containing an hydroxyl group as an initiator (available from Argonaut Technologies). The resulting polymer is phosphorylated, then oxidized to yield a solid support that contains multiple thiophosphate esters. The esters are cleaved with acid, resulting in a solid support that has (n + 1) thiophosphate moieties attached to it.

Example 3: Quenching of an aziridino compound with a thiophosphate group that is bound to a solid support

As shown in Fig. 3, Scheme 1, a nucleophilic thiophosphate group, which is bound to a solid support, attacks and quenches the aziridino compound; the aziridino compound is not only rendered inactive, it also remains bonded to the solid support through covalent bonds.

As shown in Fig. 3, Scheme 2, a solid support with (n + 1) thiophosphate groups attached is capable of quenching (n +1) aziridino

moieties.

Example 4: Quenching of oligoethyleneimine with solid-phase quenching agent containing phosphothio diester and phosphothio monoester groups

As shown in Fig. 4, thiophosphate groups on the solid support are separated by chains containing 4 atoms. When the solid support is contacted with a composition containing ethyleneimine oligomers, all of the thiophosphate groups on the solid support can react with the oligomers.

Example 5: Quenching of ethyleneimine dimer with solid phase quenching agent containing thiophosphomonoester groups

200 μl of 100 mM MOPS buffer (pH 7.0) containing 12 mM ethyleneimine dimer (2.4 μmole total) were added to 25 mg (20 μmole-equivalents of thiophosphate groups) of ArgoPore-thiophosphate solid phase support (prepared as described in Example 2). The loading of phosphothiomonoester groups on the ArgoPore-thiophosphate support was about 0.8 mmole/mg. The reaction mixture was incubated, with agitation, for 30 minutes at 23°C. Two aliquots (5 μl) of the solution were removed after 10 and 30 minutes of incubation, and the concentration of the remaining ethyleneimine dimer was determined by HPLC (Figure 5). As shown there, only 0.18% of the dimer remained in the solution after 30 minutes. This example demonstrates the ability of the support-bound thiophosphate groups to inactivate ethyleneimine dimers.

Example 6: Quenching of ethyleneimine dimer in whole CPD human blood using a solid-phase quenching agent

50 μl of 120 mM ethyleneimine dimer (EID, PEN102) in 0.25 M NaH_2PO_4 was added to 0.9 ml of whole human CPD blood (final concentration of EID was 6 mM, 6μmole total), and incubated at 23°C for 4 hours. At the

end of the 4-hour incubation period, 68 mg (50μmole-equivalents of phosphothiomonoester groups) of ArgoPore-Thiophosphate support (prepared as described in Example 2) was added. In the parallel experiment, 50 μl of 1 M $Na_2S_2O_3$ (final concentration 50 mM) was added to same amount of EID treated blood. Both samples were allowed to incubate for 2 hours at 23°C. The red blood cell (RBC) and plasma fraction of the blood were separated by centrifugation (10,000 rpm, 5 min), and the RBCs were opened by adding 9 volumes of water. The concentration of EID was determined in the RBC fraction and in the plasma fraction of the blood by HPLC (Fig. 6).

As shown in Fig. 6, both sodium thiosulfate and solid phase-bound thiophosphate groups were capable of quenching ethyleneimine dimer. After 2 hours, the plasma quenched with thiosulfate contained only 6.8 μg/ml dimer, and the red blood cells quenched with thiosulfate contained 2.2 μg/ml dimer. The solid phase quenching agent containing thiophosphate groups was even more effective. The plasma quenched with this system contained only 1.5 μg/ml dimer, and the red blood cells contained only 0.9 μg/ml dimer after 2 hours.

Example 7: Quenching of ethyleneimine dimer with solid phase quenching agent containing thiophosphodiester and thiophosphomonoester groups

200 μl of 100 mM MOPS buffer (pH 7.0) containing 12 mM ethyleneimine dimer (PEN102, 2.4 μmole total) were added to 10 mg (20 μmole-equivalent of thiophosphate groups) of ArgoGel-7BuTPh or 21 mg (20 μmole-equivalent of thiophosphate groups) of ArgoGel-3BuTPh solid phase support (shown in Fig. 4). The reaction mixture was incubated, with agitation, for 30 minutes at 23°C. Two aliquots (5 μl each) of the solution were removed after 10 and 30 minutes of incubation, and the concentration of the remaining ethyleneimine dimer was determined by HPLC (Fig. 7).

As shown there, both the ArgoGel-3BuTPh and the ArgoGel-7BuTPh systems were effective in quenching the ethyleneimine dimer. When ArgoGel-7BuTPh was used, only 11% of the dimer remained after 10 minutes, and 5.2% remained after 30 minutes. When ArgoGel-3BuTPh was used, 15.4% remained after 10 minutes, and 14.4% remained after 30 minutes.

Example 8: Preparation of a solid phase quenching agent that contains a thiophosphate group

The preparation of a solid phase quenching agent of the invention is described in Fig. 1. As shown, an aldehyde-activated Sepharose® bead (designated with a square) containing a hydroxyl group is derivatized with a phosphorylating agent. The phosphite group of the phosphorylated bead is oxidized to form a thiophosphate ester, which is cleaved with acid to provide a thiophosphate moiety. The product is a thiophosphate moiety that is attached to a Sepharose® bead through covalent bonds.

Example 9: Quenching of an aziridino compound with a thiosulfate or thiophosphate moiety that is bound to a separation moiety

A nucleophilic thiophosphate group, which is bound to a separation moiety, attacks and quenches the aziridino compound; the aziridino compound is not only rendered inactive, it also remains bonded to the quenching agent through covalent bonds.

Example 10: Separation of quenching agent and quenched inactivating agent from a biological composition by filtration

In one preferred method of the invention, the quenching moiety (containing, for example, one or more thiosulfate or thiophosphate moieties) is covalently coupled to a bead or resin, such as Sepharose® or cellulose.

The bead has particular properties which allow for its separation from the biological composition. For example, Sepharose® beads can be separated, along with the quenching agent and the quenched inactivating agent, by filtration through a filter or column with the appropriate pore size (i.e., the pores are large enough to allow passage of the biologically-important molecules, but small enough to prevent passage of the beads). Suitable filters and columns can be purchased from, for example, Millipore Corp. (Bedford, MA).

Example 11: Affinity-based separation of quenching agent and quenched inactivating agent from a biological composition by filtration

In one preferred variation, the method of the invention includes the coupling of two components. The first component includes a first quenching moiety attached to a second moiety. The second component includes a third moiety, which specifically binds to the second moiety, attached to a bead. In one example, the quenching moiety is attached to a biotin molecule, and then added to a biological composition (which had been virally inactivated with, for example, an ethyleneimine oligomer) for a length of time which allows for quenching of a viral inactivating agent. This biological composition is then passed through a column containing streptavidin-bound Sepharose®. The streptavidin specifically binds to the biotin-containing quenching agent. Hence, the biotinylated quenching agent, as well as the quenched inactivating agent, binds to the immobilized streptavidin, while the biological composition, now free of inactivating agent and quenching agent, flows through. It is understood that the streptavidin-bound Sepharose® can also be added to the biological composition, and subsequently removed through filtration, as described in Example 9, above. Similarly, other affinity-based methods can also be employed using other molecular pairs (e.g., an antigen-antibody pair,

complementary nucleic acid sequences, or the like) replacing the streptavidin-biotin pair.

Example 12: Separation of quenching agent and quenched inactivating agent from a biological composition by other methods

Those skilled in the art will recognize that there are numerous other variations which can be performed, and these variations are in the spirit of the invention. Quenching moieties bound to beads which contain iron can be separated, along with the quenched inactivating agent, by placing the biological composition in a magnetic field. Quenching moieties bound to beads having a mass substantially greater than that of biologically-important molecules can be separated by centrifugation. Moreover, these methods can be combined with the affinity-based methods described in Example 11. In one example, the quenching agent, attached to a biotin molecule, is added to a biological composition (which has been virally inactivated) for a length of time which allows for quenching of a viral inactivating agent. To this biological composition, iron-containing beads, coated with streptavidin, are added. The entire complex, including the quenched inactivating agent, is then separated from the biological composition by placing the biological composition in a magnetic field and transferring the biological composition to a second container.

In another example, quenched inactivating agent, as well as the quenching agent, can be removed by dialysis. The advantage of dialysis is that quenching agent, such as a thiosulfate or thiophosphate molecule can be removed, along with the quenched inactivating agent, whether the quenching agent is coupled to a separation moiety or not. The disadvantage is that dialysis, in contrast to the other methods of separation described herein, will

not selectively remove the inactivating and quenching agents.

All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

What is claimed is:

Claims

1. A method of inactivating a contaminant of a biological composition, said method comprising the steps of:

(a) contacting said biological composition with an inactivating agent comprising an aziridino moiety, wherein a portion of said agent reacts with and inactivates said contaminant, and a portion of said agent remains unreacted;

(b) contacting the product of step (a) with a solid support comprising at least one quenching moiety attached to said solid support through covalent bonds, under conditions and for a time sufficient to allow the unreacted agent to bond covalently to said quenching moiety; and

(c) separating said solid support and said unreacted agent from said biological composition, wherein said unreacted agent is attached to said solid support through covalent bonds.

2. A method of inactivating a contaminant of a biological composition comprising the steps of:

(a) contacting the biological composition with an inactivating agent, said agent comprising an aziridino moiety or a haloderivative salt thereof, where a portion of said agent reacts with and inactivates said contaminant, and a portion of said agent remains unreacted;

(b) contacting the product of step (a) with a quenching agent comprising a quenching moiety, under conditions and for a time sufficient to allow said inactivating agent to bond covalently to said quenching moiety; and

(c) separating said quenching agent and said quenched inactivating agent from the biological composition.

3. The method of claim 2, wherein said separating comprises the step of dialysis.

4. A method of inactivating a contaminant of a biological composition, said method comprising the steps of:

(a) contacting said biological composition with an inactivating agent comprising an aziridino moiety, wherein a portion of said agent reacts with and inactivates said contaminant, and a portion of said agent remains unreacted;

(b) contacting the product of step (a) with a quenching agent under conditions and for a time sufficient to allow said quenching agent to quench and to bond covalently to the unreacted inactivating agent;

(c) contacting the product of step (b) with a solid support comprising at least 1 aldehyde moiety covalently bonded to said solid support, under conditions and for a time sufficient to allow the quenched inactivating agent to bond covalently to said aldehyde moiety; and

(d) separating (i) said solid support, (ii) said quenched inactivating agent, and (iii) said quenching agent from said biological composition, wherein said inactivating agent and said quenching agent are attached to said solid support through covalent bonds.

5. The method of claim 4, wherein said solid support comprises at least 2 mmol/g aldehyde moieties.

6. The method of claim 4, wherein said solid support comprises at least 100 mmol/g aldehyde moieties.

7. A method of inactivating a contaminant of a biological composition comprising the steps of:

(a) contacting the biological composition with an inactivating agent, said agent comprising an aziridino moiety or a haloderivative salt thereof, where a portion of said agent reacts with and inactivates said contaminant, and

a portion of said agent remains unreacted;

(b) contacting the product of step (a) with a quenching agent comprising a quenching moiety attached to a separation moiety through covalent bonds under conditions and for a time sufficient to allow said inactivating agent to bond covalently to said quenching moiety; and

(c) separating said separation moiety, said quenching moiety and said quenched inactivating agent from the biological composition.

8. A method of removing a viral inactivating agent from a biological composition comprising the steps of:

(a) contacting said inactivating agent with a quenching moiety that is coupled to a separation moiety; and

(b) removing said inactivating agent, said quenching moiety, and said separation moiety from the biological composition.

9. The method of claim 7, wherein said quenching agent further comprises a reporter moiety selected from the group consisting of a UV adsorbing moiety and a fluorescent moiety.

10. The method of claim 8, wherein step (a) comprises contacting said inactivating agent with said quenching moiety under conditions and for a time sufficient to allow covalent bonds to form between said inactivating agent and said quenching moiety.

11. The method of claim 1, 2, 7, or 8, wherein said quenching moiety comprises a nucleophilic moiety selected from the group consisting of a thiophosphate moiety and a thiosulfate moiety.

12. The method of claim 11, wherein said thiophosphate moiety is part of an internucleotide linkage of an oligonucleotide sequence.

13. A method of removing a viral inactivating agent from a biological composition, said method comprising the steps of:

(a) contacting said inactivating agent with a quenching agent that is attached to a solid support through covalent bonds; and

(b) removing said inactivating agent, said quenching agent, and said solid support from said biological composition.

14. The method of claim 13, wherein step (a) comprises contacting said inactivating agent with said quenching agent under conditions and for a time sufficient to allow covalent bonds to form between said inactivating agent and said quenching agent.

15. The method of claim 4 or 13, wherein said quenching agent comprises a nucleophilic moiety.

16. The method of claim 4 or 13, wherein said quenching agent comprises a thiophosphate moiety or a thiosulfate moiety.

17. The method of claim 1, 2, 4, 7, 8, or 13, wherein said inactivating agent is selected from the group consisting of ethyleneimine, N-acetylethyleneimine, an ethyleneimine oligomer, a haloderivative salt of ethyleneimine, and a haloderivative salt of an ethyleneimine oligomer.

18. The method of claim 17, wherein said inactivating agent is N-acetylethyleneimine.

19. The method of claim 1, 2, 4, 7, 8, or 13, wherein said biological composition is selected from the group consisting of whole mammalian blood, purified or partially purified blood proteins, blood cell proteins, milk, saliva, blood plasma, platelet-rich plasma, a plasma concentrate, a precipitate from any fractionation of plasma, a supernatant from any fractionation of plasma, a serum, a cryoprecipitate, a cryosupernatant, a cell lysate, a mammalian cell culture, a mammalian culture supernatant, a placental extract, a product of fermentation, a platelet concentrate, a leukocyte concentrate, semen, red blood cells, and a recombinant protein-containing composition produced in a transgenic mammal.

20. The method of claim 19, wherein said biological composition is whole human blood or human blood plasma.

21. The method of claim 1, 2, 4, or 7, wherein said contaminant is a virus.

22. A method of quenching an electrophile, said method comprising contacting said electrophile with a solid support comprising at least one thiophosphate moiety attached to said solid support through covalent bonds, under conditions and for a time sufficient to allow said electrophile to bond covalently to said thiophosphate moiety.

23. The method of claim 22, wherein said electrophile comprises an aziridino moiety or a haloderivative salt thereof.

24. The method of claim 22, wherein said solid support comprises at

least 2 mmol/g thiophosphate moieties.

25. The method of claim 24, wherein said solid support comprises at least 100 mmol/g thiophosphate moieties.

26. The method of claim 24, wherein a plurality of said thiophosphate moieties are substituted with at least one C_{1-12} saturated or unsaturated hydrocarbon skeleton that is unsubstituted or has between 1 and 4, inclusive, substituents, independently selected from the group consisting of hydroxyl, amino, cyano, and azido groups.

27. A method of quenching an electrophile comprising contacting said electrophile with a quenching agent comprising a thiosulfate or thiophosphate moiety attached to a separation moiety, under conditions and for a time sufficient to allow said electrophile to bond covalently to said thiosulfate or thiophosphate moiety.

28. The method of claim 27, wherein said electrophile comprises an aziridino moiety or a haloderivative salt thereof.

29. The method of claim 28, wherein said electrophile is selected from the group consisting of ethyleneimine, N-acetylethyleneimine, an ethyleneimine oligomer, a haloderivative salt of ethyleneimine, and a haloderivative salt of an ethyleneimine oligomer.

30. The method of claim 29, wherein said electrophile is N-acetylethyleneimine.

31. The method of claim 27, wherein said quenching agent further comprises a reporter moiety selected from the group consisting of a UV adsorbing moiety and a fluorescent moiety.

32. The method of claim 7, 8, or 27, wherein said separation moiety is selected from the group consisting of a bead, a resin, an antibody, and a biotin molecule.

33. A compound comprising:

(a) a separation moiety; and

(b) a thiosulfate or a thiophosphate moiety.

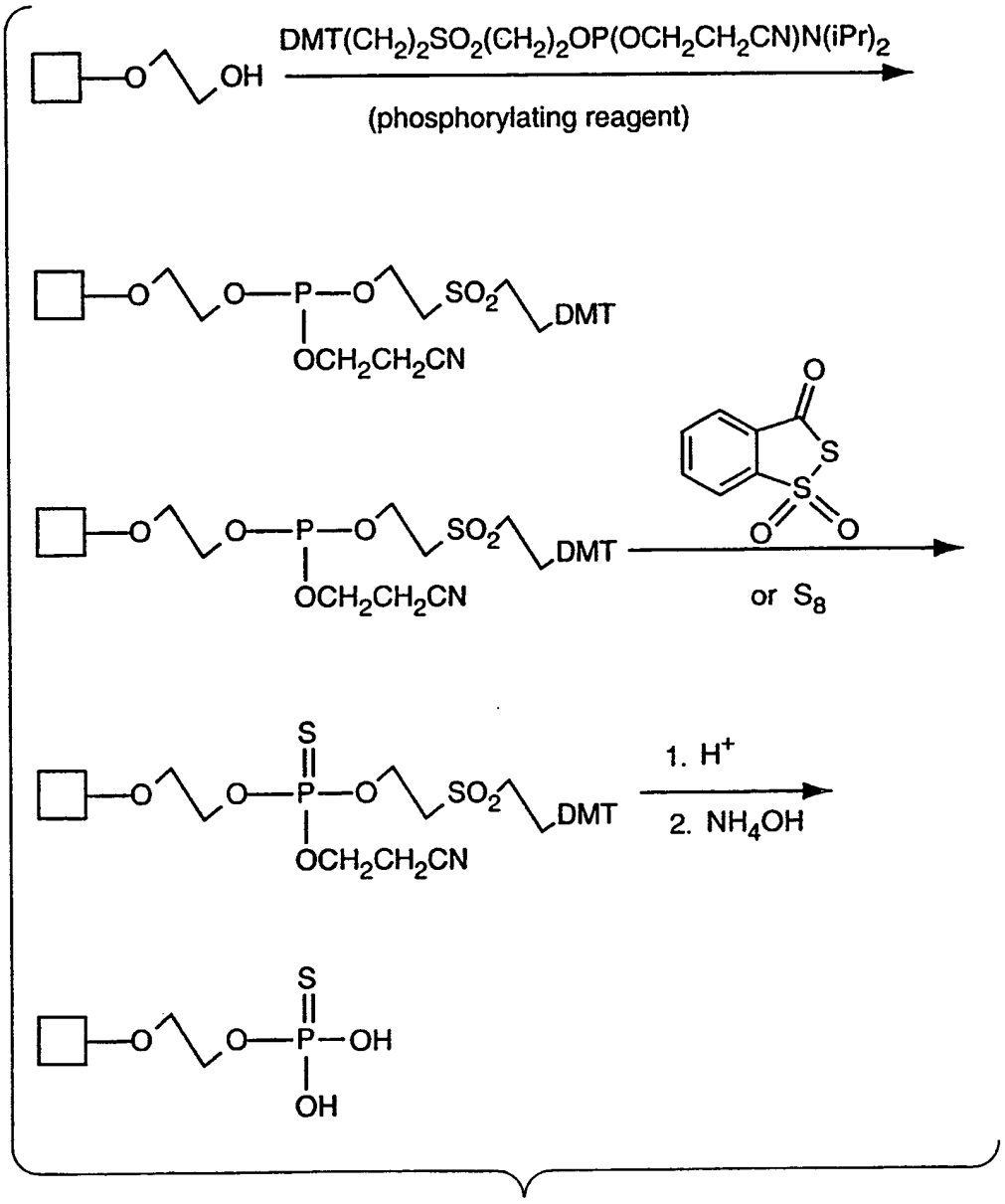
34. The compound of claim 33, wherein said separation moiety is selected from the group consisting of a bead, a resin, an antibody, and a biotin molecule.

35. The compound of claim 33, further comprising a reporter moiety, selected from the group consisting of a UV adsorbing moiety and a fluorescent moiety.

36. The compound of claim 33, wherein said thiophosphate moiety is part of an internucleotide linkage of an oligonucleotide sequence.

37. The use of a quenching agent attached to a solid support for the removal of inactivating agent from a biological composition.

38. The use of a compound comprising a quenching moiety and a separation moiety for the removal of inactivating agent from a biological composition.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(phosphorylating reagent)
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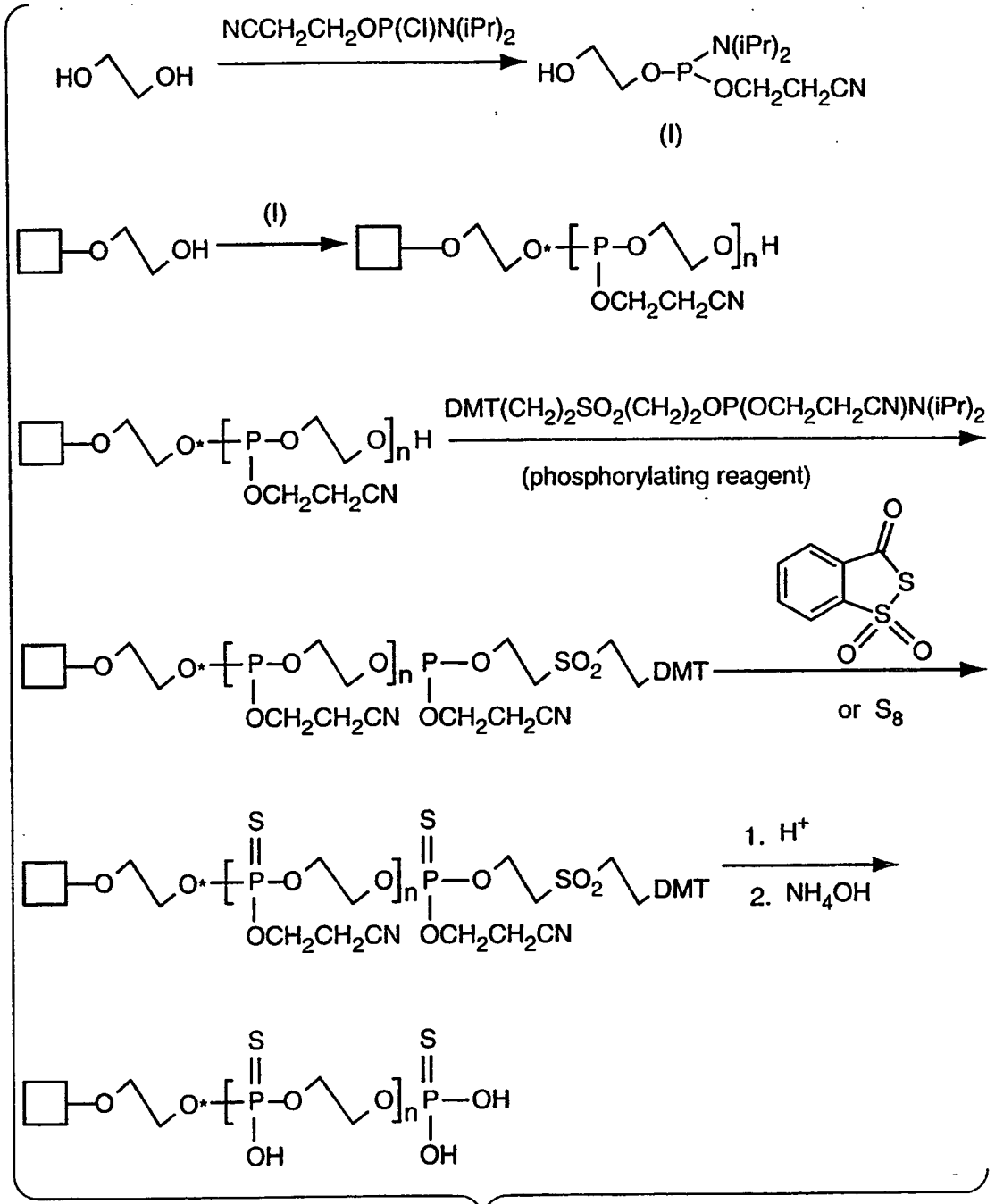
DMT
OCH2CH2CN
or S8
1. H+
2. NH4OH

Fig. 1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HO O–P N(iPr)2 OCH2CH2CN
(I)
(I)
OH
O O* P–O O]n H
OCH2CH2CN
DMT(CH2)2SO2(CH2)2OP(OCH2CH2CN)N(iPr)2
(phosphorylating reagent)
SO2 DMT
or S8
1. H+
2. NH4OH
S
OH

Fig. 2

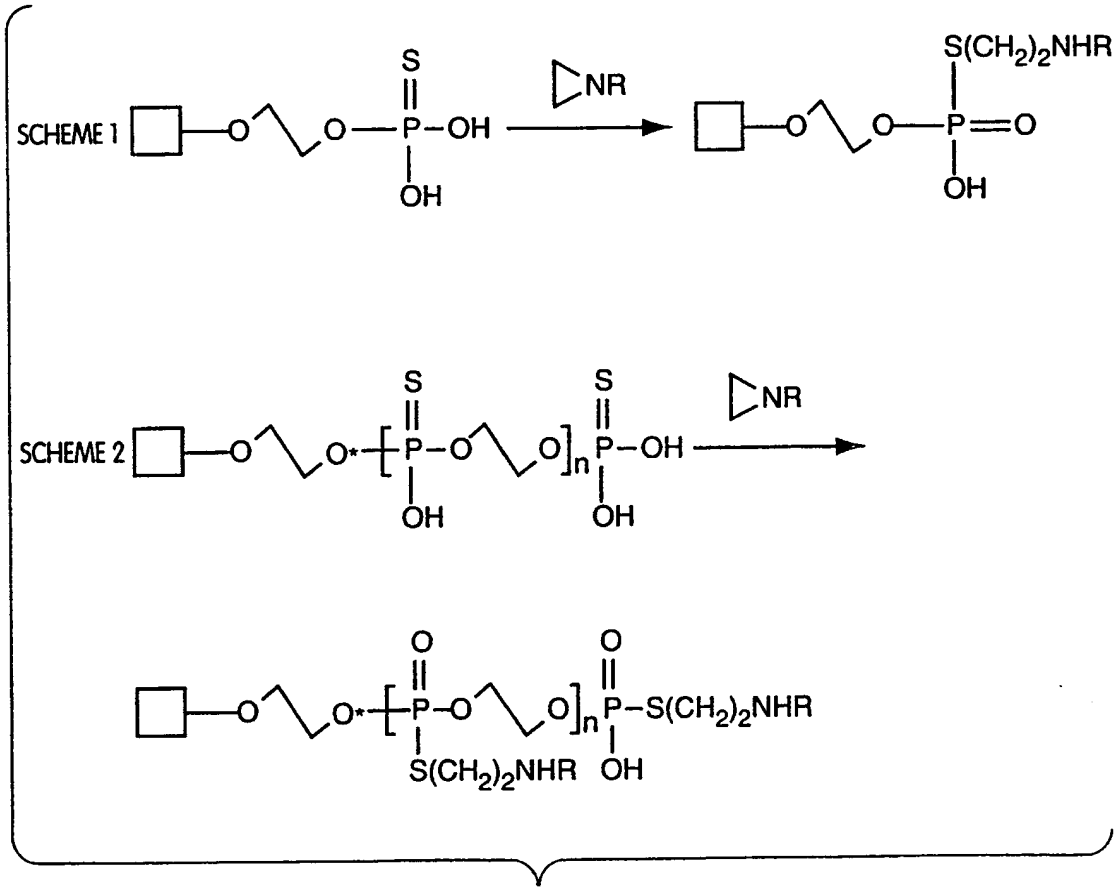


Fig. 3

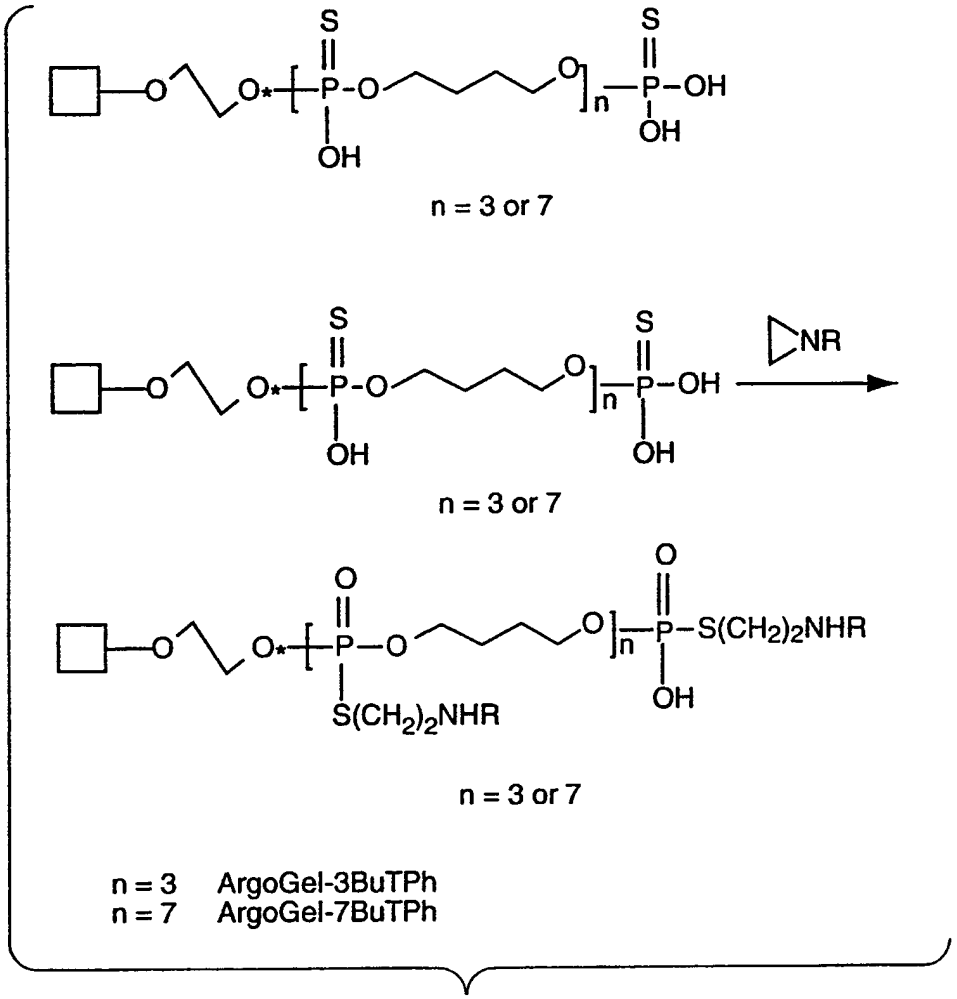
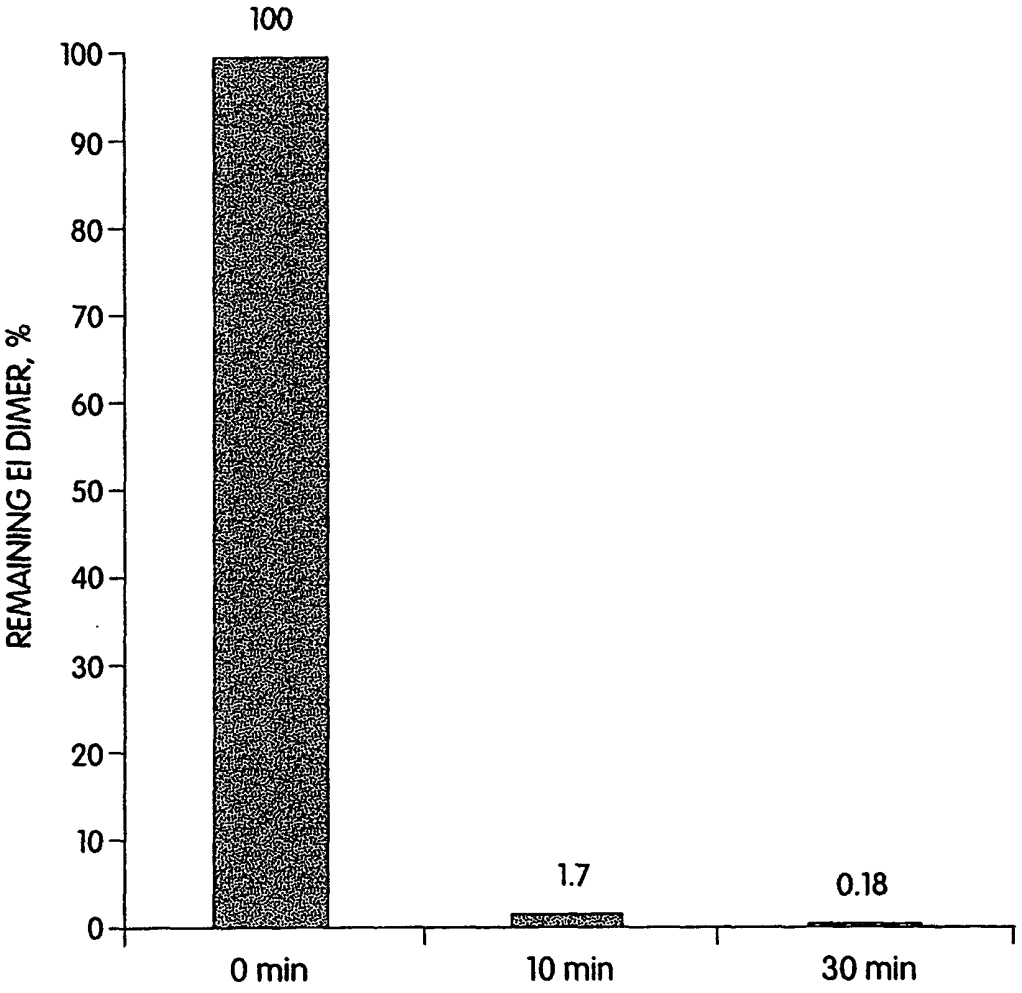


Fig. 4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100
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80
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60
50
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30
20
10
0
REMAINING EI DIMER, %
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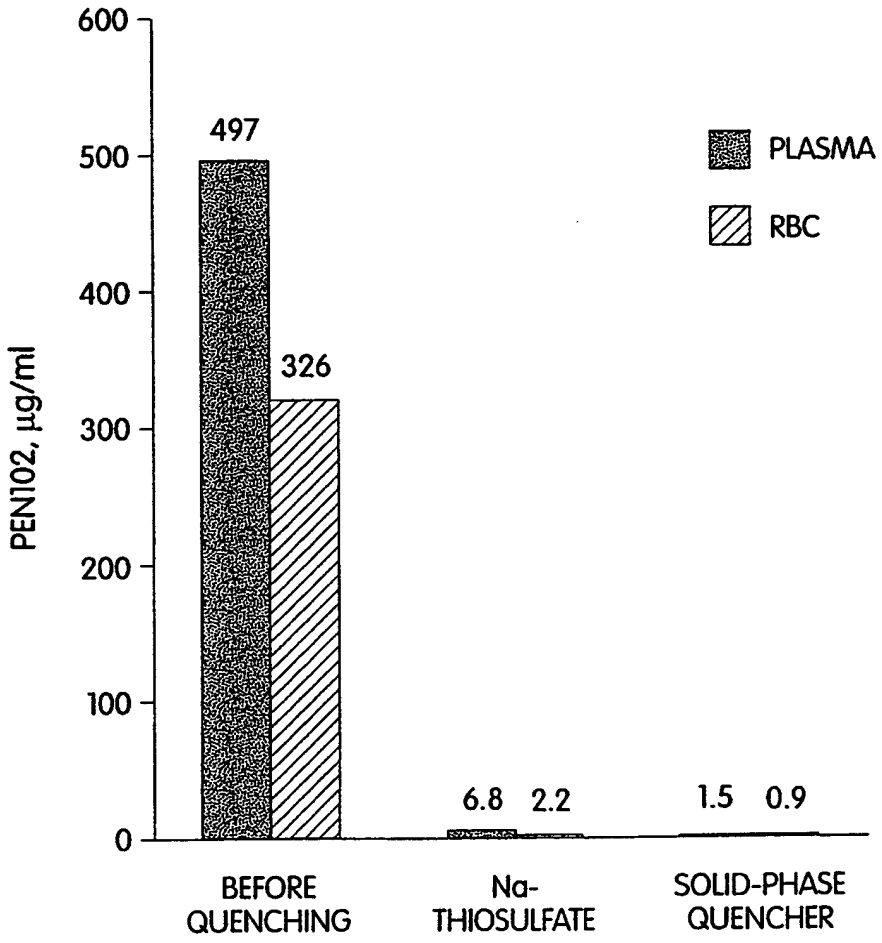
10 min
30 min

Fig. 5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400
300
200
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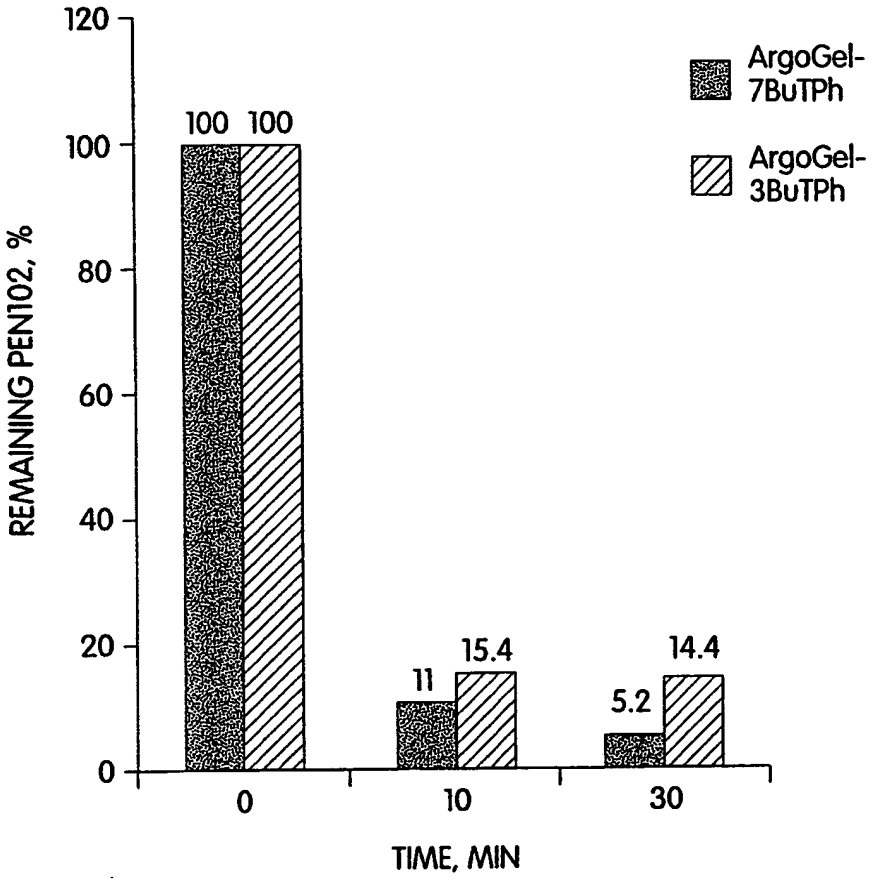
PLASMA
RBC
BEFORE QUENCHING
Na-THIOSULFATE
SOLID-PHASE QUENCHER

Fig. 6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80
60
40
20
0
REMAINING PEN102, %
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5.2
14.4
0
10
30
TIME, MIN
ArgoGel-7BuTPh
ArgoGel-3BuTPh

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21769

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 35/14; C12N 7/06
US CL :435/238; 422/28; 548/314.7

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/238; 422/28; 548/314.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,581,368 A (AHMED et al) 08 April 1986, see entire document.	1-38
Y	US 4,757,148 A (AHMED et al) 12 July 1988, see entire document.	1-38

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 DECEMBER 1999	Date of mailing of the international search report 01 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer L. BLAINE LANKFORD Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*